

# CANCER AND AGEING: RIVAL DEMONS?

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Organisms with renewable tissues use a network of genetic pathways and cellular responses to prevent cancer. The main mammalian tumour-suppressor pathways evolved from ancient mechanisms that, in simple post-mitotic organisms, act predominantly to regulate embryogenesis or to protect the germline. The shift from developmental and/or germline maintenance in simple organisms to somatic maintenance in complex organisms might have evolved at a cost. Recent evidence indicates that some mammalian tumour-suppressor mechanisms contribute to ageing. How might this have happened, and what are its implications for our ability to control cancer and ageing?

## COMPLEX ORGANISMS

Multicellular organisms that are composed of both post-mitotic and renewable (mitotic) somatic tissues.

## SIMPLE ORGANISMS

Multicellular organisms that are composed entirely or largely of post-mitotic somatic cells.

## CARETAKERS

Tumour-suppressor genes or proteins that act to protect the genome from damage or mutations. Many caretaker genes encode proteins that recognize or repair DNA damage.

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Cancer afflicts COMPLEX ORGANISMS, such as mice and humans, almost inevitably as they approach middle and old age<sup>1–3</sup> (FIG. 1; BOX 1). This is not the case for all organisms, however; the SIMPLE model invertebrate organisms *Caenorhabditis elegans* and *Drosophila melanogaster*, for example, do not develop cancer. So, how prevalent is cancer among animals, and what distinguishes organisms that develop cancer from those that do not?

## Tumour-suppressor mechanisms

Whether multicellular organisms are subject to cancer depends to some extent on whether they are simple or complex. An important distinction between simple and complex organisms is that complex organisms have renewable tissues that are essential for viability, and this might explain their susceptibility to cancer. Renewable tissues allow adult organisms to replace cells that are lost through stochastic, pathological or catastrophic damage, or through differentiation. However, the cell proliferation that occurs in renewing tissues puts the genome at great risk for acquiring and propagating mutations that can confer malignant characteristics on cells (BOX 1). So, it seems that, as complex organisms with renewable tissues evolved, so did the risk of cancer.

Complex organisms evolved strategies — tumour-suppressor mechanisms — to suppress the development of cancer, at least through the period of sexual maturity and reproduction (young adulthood). These mechanisms

have been studied extensively, primarily in mice and humans. It is clear that at least two main strategies evolved to suppress cancer<sup>4</sup>. One mechanism uses CARETAKER proteins to protect the genome from acquiring potentially oncogenic mutations. The other uses GATEKEEPER proteins to eliminate or prevent the growth of potential cancer cells (FIG. 2). Both mechanisms evolved from ancestral genetic pathways, elements of which were, and still are, present in simple organisms that do not develop cancer.

An important distinction between caretaker and gatekeeper tumour suppressors is that caretakers generally operate within the context of the cell, whereas gatekeepers operate within the context of the tissue or organism. That is, caretakers act to preserve cellular integrity and survival, whereas gatekeepers cause cell death or loss of cell-division potential for the good of the organism. Consistent with this general distinction, the origin of many (but certainly not all) caretakers can be traced back to genes that are present in simple single-celled organisms. So, many caretaker genes encode proteins that participate in evolutionarily conserved, genomic maintenance functions, such as DNA-repair pathways. These include RECQ-like helicases, components of the NUCLEOTIDE EXCISION REPAIR pathway and TELOMERE maintenance proteins. By contrast, many gatekeeper tumour-suppressor genes do not exist in single-celled organisms, but appear

## Summary

- **Cancer is a problem that affects organisms with renewable tissues; these have evolved tumour-suppressor mechanisms to suppress the development of cancer.**
- **Tumour-suppressor genes act to prevent or repair genomic damage (caretakers), or inhibit the propagation of potential cancer cells (gatekeepers) by permanently arresting their growth (cellular senescence) or inducing cell death (apoptosis).**
- **Some caretaker tumour suppressors seem to postpone the development of ageing phenotypes, and so are also longevity-assurance genes.**
- **The gatekeeper tumour-suppressor mechanisms (apoptosis and cellular senescence), by contrast, might promote certain ageing phenotypes.**
- **Apoptosis and cellular senescence are controlled by the p53 and RB tumour-suppressor pathways, components of which are evolutionarily conserved among multicellular organisms.**
- **The evolutionary hypothesis of antagonistic pleiotropy predicts that some processes that benefit young organisms (by suppressing cancer, for example) can have detrimental effects later in life and would therefore contribute to ageing.**
- **Both apoptosis and cellular senescence might be antagonistically pleiotropic, promoting ageing by exhausting progenitor or stem cells. Additionally, senescent cells secrete factors that can disrupt tissue integrity and function, and even promote the progression of late-life cancers.**
- **Recent studies on p53 provide a molecular basis for how tumour suppression and ageing might be intertwined.**

## GATEKEEPERS

Tumour-suppressor genes or proteins that regulate cellular responses that prevent the survival or proliferation of potential cancer cells. These responses are known as apoptosis and cellular senescence, respectively.

## NUCLEOTIDE EXCISION REPAIR

A DNA-repair pathway that removes and replaces damaged nucleotides, particularly those that distort the DNA helix.

## TELOMERES

The DNA–protein structure that stabilizes the ends of linear chromosomes and protects them from degradation or fusion. In vertebrates, telomeres are composed of several-kilobase pairs of the sequence TTTAGGG and several associated proteins.

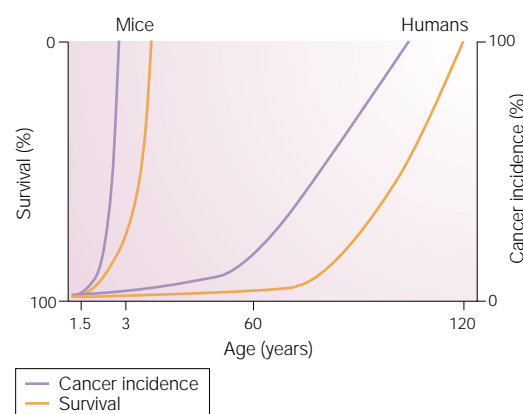
## APOPTOSIS

Ordered, genetically programmed cell death triggered by both physiological stimuli and cellular damage. Apoptosis avoids cell lysis and subsequent inflammation.

## CELLULAR SENESCENCE

The essentially irreversible loss of cell division potential and the associated functional changes that are triggered by damage and other potential cancer-causing stimuli.

with the evolution of multicellular organisms. Examples include the genes that encode the **p53** and **RB** proteins, which control the cellular responses of APOPTOSIS and CELLULAR SENESCENCE.



**Figure 1 | Cancer increases with ageing.** Cancer incidence (although not necessarily death from cancer) rises exponentially with age, beginning at about the mid-point of the maximum lifespan of the species<sup>1–3</sup>. So, mice, which have a maximum lifespan of 3–4 years, generally develop cancer at 18–24 months of age. By contrast, most human cancers develop after 50–60 years, or halfway through the 100–120-year maximum lifespan of humans. These are, of course, average or general trends. Genes (intra-species variants, also known as polymorphisms, and probably inter-species variants or homologues) can strongly influence the probability of cancer developing in a particular organism or a particular tissue. Similarly, cancer incidence is strongly influenced by external or environmental factors, such as exposure to mutagens or toxins, or conditions that stimulate chronic cell proliferation (for example, chronic inflammation or lytic infections).

## Tumour suppression and longevity

Tumour-suppressor genes prevent premature death from cancer, so it stands to reason that they should also be classified as LONGEVITY-assurance genes — genes that slow the AGEING process and promote the health and survival of adult organisms<sup>5</sup>. Indeed, this is probably the case for caretaker tumour suppressors, but recent findings raise the possibility that some gatekeeper tumour suppressors can actually contribute to the development of AGEING PHENOTYPES in complex organisms. What are the relationships between tumour-suppressor and longevity-assurance genes, and how might tumour suppressors have both beneficial (preventing cancer) and detrimental (promoting ageing) effects?

Regardless of whether tumour suppressors are caretakers or gatekeepers, inactivating mutations increase the risk for developing cancer. Therefore, all tumour suppressors should directly promote the longevity of complex multicellular organisms by curtailing the development of malignant tumours, and loss of tumour-suppressor function should shorten the average lifespan by increasing the incidence of cancer.

Numerous lines of evidence, particularly from mouse models and human genetics, support this obvious and direct relationship between tumour-suppressor mechanisms and longevity<sup>6–16</sup>. However, recent findings indicate that a more complex relationship exists — tumour-suppressor mechanisms can influence ageing, which ultimately limits longevity. The links between tumour suppression and ageing are twofold.

First, some tumour-suppressor mechanisms not only curtail cancer, but also seem to retard the appearance of specific ageing phenotypes. Recent findings show that defects in certain DNA-repair pathways increase both the incidence of cancer and the rate at which specific ageing phenotypes develop<sup>17</sup>. These findings support the idea that DNA damage and loss of genomic integrity in somatic cells can contribute to ageing phenotypes other than cancer<sup>18</sup>. So, some caretaker tumour suppressors might act as longevity-assurance genes, independently of their role in tumour suppression.

Conversely, recent evidence indicates that other tumour-suppressor mechanisms, particularly the gatekeeper mechanisms of apoptosis and cellular senescence, dually suppress the development of cancer and promote the development of specific ageing phenotypes. These findings raise the possibility that some tumour-suppressor genes show ANTAGONISTIC PLEIOTROPY<sup>19,20</sup>, and therefore contribute to ageing. Moreover, when caretaker mechanisms fail, the ageing phenotypes that develop might derive not only from the loss of genomic integrity, but also from the apoptosis and/or cellular senescence that can occur in response to the accumulated damage. So, the caretaker and gatekeeper tumour-suppressor mechanisms can interact. Needless to say, this new appreciation has important implications for our prospects of preventing and treating cancer, as well as other pathologies that are associated with ageing.

## Box 1 | Characteristics and causes of cancer

**What is cancer?**

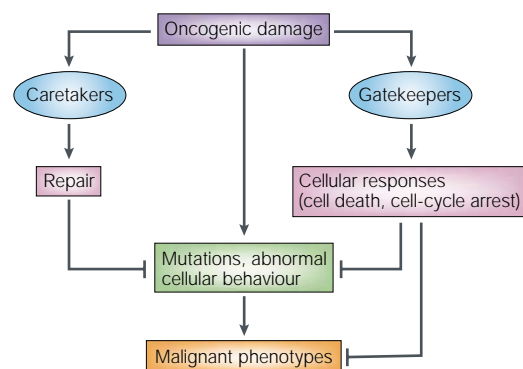
Cancer is a cellular phenomenon that occurs because cells acquire certain abnormal properties. These properties, or malignant phenotypes, allow cells to form multicellular masses that have the potential to kill the organism.

The malignant phenotypes acquired by cancer cells can be summarized as follows:

- loss of growth control (self-sustaining growth signals, insensitivity to inhibitory signals);
- resistance to apoptosis or programmed cell death;
- an extended or indefinite replicative lifespan (replicative immortality);
- ability to attract or create a bloody supply (angiogenesis);
- ability to invade the surrounding tissue;
- ability to colonize and survive in an ectopic environment (metastasis).

**What causes cancer?**

Several decades of research have shown that at least two processes, both of which occur more frequently with age, are essential for cancer development. The first is the acquisition of mutations<sup>2,8,15,131</sup>. Cancer-causing mutations, directly or indirectly, confer on cells the malignant properties described above. However, in many cases, oncogenic mutations alone might not be sufficient to form a malignant tumour<sup>132–135</sup>. It has long been appreciated that normal tissues can often prevent potential tumour cells from proliferating or expressing malignant phenotypes<sup>136</sup>. More recent data show that the tissue microenvironment, which includes its structure and cellular, extracellular and hormonal/cytokine/growth-factor composition, is another determinant of whether, and to what extent, potential cancer cells can express their malignant phenotypes<sup>136–138</sup>.



**Figure 2 | Tumour-suppressor mechanisms.** Oncogenic damage engages tumour-suppressor mechanisms to suppress the development of malignant tumours, and refers to any intracellular- or extracellular event that can lead to a malignant phenotype. It includes chemicals, radiation and other events that can ultimately lead to mutations, as well as damage that alters normal cell–cell or cell–tissue communication, control of gene expression or signal transduction, which can cause abnormal cellular behaviour (such as inappropriate cellular growth or movement within tissues). Broadly considered, caretaker tumour suppressors repair oncogenic damage. Gatekeeper tumour suppressors, by contrast, trigger cellular responses to oncogenic damage, the most important of which are cell death (apoptosis) and cell-cycle arrest, which can be transient or permanent (cellular senescence). Although there is no direct proof, as yet, gatekeepers are presumed to function when cells sense that the damage cannot be repaired, or when the damage is not repaired, whereupon the ensuing cell death or growth arrest prevents the survival or propagation of abnormally behaving cells.

**LONGEVITY**

Average or maximum lifespan of a cohort of organisms.

**AGEING**

The decline in organismal fitness that occurs with increasing age.

**AGEING PHENOTYPES**

The specific physiological manifestations of ageing.

**ANTAGONISTIC PLEIOTROPY**

The hypothesis that genes or processes that were selected to benefit the health and fitness of young organisms can have unselected deleterious effects that are manifest in older organisms and thereby contribute to ageing.

Genome maintenance and longevity assurance  
Many caretakers curtail cancer by preventing or repairing genomic damage, thereby directly suppressing the acquisition of oncogenic mutations. In many (but not all) cases, caretaker genes evolved from genetic pathways that exist in all free-living organisms, from bacteria to humans. In principle, these genes could encode proteins that prevent DNA damage (for example, antioxidant enzymes). However, most of those identified so far encode proteins that are important in repairing DNA damage or maintaining genomic integrity.

One interesting example of an ancestral caretaker is the DNA helicase RECQ. It was first identified in the bacterium *Escherichia coli*<sup>21</sup>, in which it is important for resolving certain types of DNA damage by recombination. RECQ-like genes have also been found in eukaryotes. Budding and fission yeast each contain one such gene, and in both cases (*SGS1* in *Saccharomyces cerevisiae* and *RQH1* in *Schizosaccharomyces pombe*) the RECQ-like protein seems to be particularly important for repairing damage that occurs during DNA replication<sup>22–24</sup>. Complex eukaryotes have several RECQ-like helicases, indicating that these proteins took on different functions as organismal complexity evolved. In all organisms studied, defects in RECQ-like helicases result in genomic mutations — typically chromosomal aberrations — and instability<sup>24–26</sup>.

Humans have five RECQ-like helicases: RECQ1, BLM, WRN, RTS and RECQ5. Three of these — BLM, WRN and RTS — give rise to the hereditary Bloom, Werner and Rothmund–Thomson syndromes, respectively, when defective<sup>25,27–33</sup>. BLM, WRN and RTS are thought to participate in DNA-repair pathways, particularly those that repair double-strand DNA breaks, and their loss results in chromosomal deletions, translocations and other aberrations, which are a cause and hallmark of cancer. Indeed, Bloom, Werner and Rothmund–Thomson syndromes are all characterized by a high incidence of cancer, indicating that the respective genes are caretaker tumour suppressors<sup>34</sup>. However, each syndrome is also characterized by additional pathologies — some of which are common to more than one syndrome (such as type II diabetes in Bloom syndrome and Werner syndrome), whereas others are syndrome-specific (such as cardiovascular disease in Werner syndrome and skeletal abnormalities in Rothmund–Thomson syndrome).

Werner syndrome is unique among the RECQ-like helicase diseases, and indeed among all human hereditary diseases, in that it is the clearest example of an adult-onset premature-ageing syndrome<sup>25,27,28,31,33,35</sup>. Individuals with Werner syndrome are essentially asymptomatic for the first decade of life. Thereafter, they develop — at an accelerated rate — many benign and pathological phenotypes that are associated with ageing. These include the thinning and greying of hair, thinning and wrinkling of skin, bilateral cataracts, type II diabetes, osteoporosis, cardiovascular disease and cancer. Individuals with Werner syndrome generally die in the fifth decade of life from cancer or cardiovascular disease<sup>27,28,33</sup>. Cells from individuals with Werner

syndrome are genomically unstable — accumulating large deletions and chromosomal translocations at an abnormally high frequency<sup>36–39</sup>. The mutation-prone phenotype of Werner syndrome cells and the cancer-prone phenotype of Werner syndrome individuals argues that *WRN* is a caretaker tumour-suppressor gene<sup>25,31,40</sup>; however, *WRN* must also suppress the development of ageing phenotypes that are unrelated to cancer. Loss of genomic integrity can therefore lead to age-associated pathologies other than cancer; moreover, some caretaker tumour suppressors, such as *WRN*, are longevity-assurance genes, independent of their tumour-suppressor functions. The mutations that accumulate in individuals with Werner syndrome could be responsible for both the cancer and ageing phenotypes. Alternatively, the ageing phenotypes in Werner syndrome could result from the cellular responses of apoptosis or senescence to the unrepaired or poorly repaired damage that can accumulate in the cells.

Many other DNA-repair systems exist in mammals, including MISMATCH, BASE EXCISION and nucleotide excision repair, by NON-HOMOLOGOUS END-JOINING REPAIR and HOMOLOGOUS RECOMBINATIONAL REPAIR. Some of these systems have also been conserved throughout evolution. In complex organisms, defects in key components of these systems cause cancer-prone syndromes, indicating that they are tumour suppressors<sup>41–48</sup>. Interestingly, a subset of such defects also accelerates ageing. For example, targeted disruption of *Ku80* — a key component of non-homologous end-joining repair — in mice accelerates the appearance of preneoplastic nodules, but also accelerates the development of osteoporosis, skin atrophy and other signs of ageing<sup>49</sup>. Another interesting example is a specific defect in nucleotide excision repair. Defects in any one of seven proteins (*XPA-XPG*) that participate in this DNA-repair system cause XERODERMA PIGMENTOSUM in humans<sup>42,47,50</sup>; however, a specific mutation in *XPD* causes a different syndrome — trichothiodystrophy (TTD) or brittle-hair disorder. In humans and a genetically engineered mouse model, TTD does not result in cancer predisposition. Rather, TTD presents with several features of premature ageing<sup>51</sup>. Individuals with TTD not only have defective nucleotide excision repair, but also have impaired transcription, which can cause apoptosis. So, the premature ageing of individuals with TTD might be due to genomic deterioration, excessive apoptosis, or both. Likewise, mice carrying a truncation mutation in the *Brca1* gene — which is believed to be important in repairing DNA damage during replication<sup>52</sup> — are cancer prone, as expected, but also show signs of premature ageing<sup>53</sup>. Cells from these mice are prone to undergo cellular senescence. These mice probably develop cancer because they acquire mutations as a consequence of suboptimal repair during DNA replication. Their premature ageing, on the other hand, might be due to loss of genomic integrity, excessive cellular senescence, or both.

In these cases, it seems that caretaker tumour suppressors fulfil two functions in complex organisms, both of which promote organismal longevity. They suppress the development of cancer, but they also suppress the development of phenotypes that are associated with ageing.

Tumour-suppressor mechanisms and ageing

In contrast to caretaker tumour suppressors, gatekeeper tumour suppressors regulate or effect cellular responses to events that are potentially oncogenic. These include genomic damage, epigenetic changes that derange gene expression or disruptions in the cellular microenvironment that alter cellular behaviour (BOX 1). Gatekeeper tumour suppressors typically participate in apoptosis or an arrest of cell proliferation/growth. The growth arrest can be either transient, which is thought to allow time for repair, or permanent. The permanent arrest is known as cellular senescence, or the senescence response.

**Apoptosis and tumour suppression.** Apoptosis causes cells to die in a rapid, regulated manner, whereby the cellular contents are systematically crosslinked and then removed by scavenging cells. Apoptosis ensures that cells die without releasing destructive degradative enzymes or triggering inflammatory reactions, which occurs when cells die by lysis or NECROSIS<sup>54,55</sup>.

In all multicellular eukaryotes examined, apoptosis functions during embryonic development to eliminate excess cells or cells that have not made proper intercellular connections<sup>56</sup>. It also eliminates damaged cells from the germline<sup>57</sup>. Apoptosis is essential for embryonic development and germline maintenance in both simple and complex eukaryotic organisms. Moreover, there is sequence and functional homology among the regulators and effectors of apoptosis, from simple organisms, such as *C. elegans*, to complex organisms, such as humans. Although certain features of apoptosis have been reported in aged yeast<sup>58</sup>, these features only superficially resemble those of apoptotic cells from multicellular organisms (and it is not clear why programmed cell death would have evolved in a single-celled organism).

In complex organisms, apoptosis is also essential for the homeostatic maintenance of renewable tissues in adults. In such tissues, an important function of apoptosis is to eliminate dysfunctional or damaged — and therefore potentially oncogenic — cells. There is little doubt that apoptosis is an important defence against cancer<sup>59,60</sup>. Cancer cells almost invariably acquire mutations that allow them to evade normal signals and mechanisms that cause apoptotic cell death<sup>10</sup> (BOX 1). Moreover, mice that are engineered to carry (non-embryonic lethal) mutations that compromise the ability of cells to die by apoptosis are generally cancer prone<sup>9,14,16</sup>.

**Cellular senescence and tumour suppression.** Cellular senescence or the senescence response causes cells to arrest proliferation, essentially irreversibly, in response to stimuli that put them at risk for malignant transformation<sup>61</sup>. Cellular senescence was first identified as replicative senescence — the process that limits the replicative lifespan of cells, now known to be caused by the shortening and consequent dysfunction of telomeres in human cells. Several other stimuli have since been shown to arrest cells with a senescent phenotype, rapidly and without extensive cell division; these stimuli are said to induce 'premature' senescence<sup>62,63</sup>. They include DNA damage,

#### MISMATCH REPAIR

A DNA-repair pathway that removes and replaces nucleotides that have been misrepaired by DNA polymerases during DNA replication.

#### BASE EXCISION REPAIR

A DNA-repair pathway that excises and replaces damaged DNA bases.

#### NON-HOMOLOGOUS END-JOINING REPAIR

A relatively error-prone pathway that repairs double-strand breaks by ligating non-homologous DNA ends.

#### HOMOLOGOUS RECOMBINATIONAL REPAIR

A relatively error-free pathway that repairs DNA double-strand breaks using an undamaged sister chromatid or homologous chromosome as a template.

#### XERODERMA PIGMENTOSUM

A group of cancer-prone syndromes in humans that are caused by defects in the nucleotide excision repair genes.

#### NECROSIS

Passive or unregulated cell death, in which cells lyse and deposit degradative and antigenic cell constituents into the surrounding tissue. Necrotic cell death, in contrast to apoptosis, often provokes an inflammation reaction.

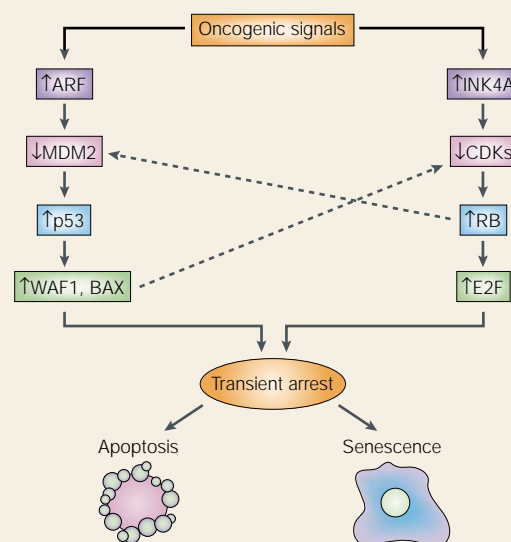


## Box 2 | The p53 and RB tumour-suppressor pathways

p53 and RB are at the heart of the two main tumour-suppressor pathways that control cellular responses to potentially oncogenic stimuli (see figure). Each pathway consists of several upstream regulators and downstream effectors. For simplicity, only four main components in each pathway are shown. Similarly, the pathways interact at several points, two of which are shown<sup>96,101,139–141</sup>.

In the p53 pathway, signals such as DNA damage induce the ARF (also known as p14 in humans and p19 in mice) product of the *CDKN2A* locus. ARF increases p53 levels by sequestering MDM2, which facilitates the degradation and inactivation of p53. p53 has both transactivation and transrepression activity, and so controls the transcription of numerous genes. Among the p53 target genes are WAF1, an inhibitor of cyclin-dependent protein kinases (CDKs) that, among other activities, causes cell-cycle arrest, and BAX, which promotes apoptotic cell death.

In the RB pathway, stress signals such as oncogenes induce INK4A, the other product of the *CDKN2A* locus. INK4A inhibits CDKs that phosphorylate, and therefore inactivate, RB during the G1 phase of the cell cycle. RB also controls the expression of numerous genes, although it does so primarily by recruiting transcription factors and chromatin remodelling proteins. One downstream consequence of RB activity is the inhibition of E2F activity, which is important for the transcription of several genes that are required for progression through the G1 and S phases of the cell cycle. RB also regulates p53 activity through a trimeric p53–MDM2–RB complex<sup>139</sup>.



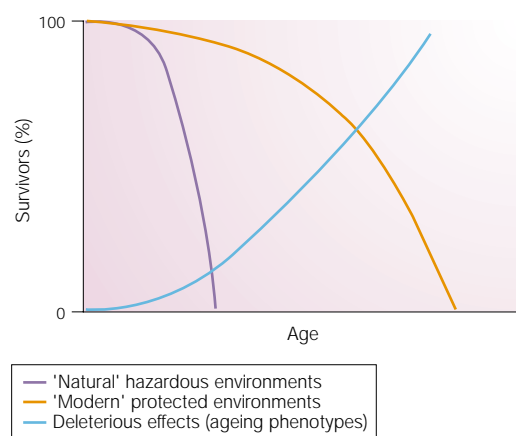
the expression of certain oncogenes and disruptions to chromatin structure<sup>61,64,65</sup>. In contrast to apoptosis, cellular senescence does not eliminate dysfunctional or damaged cells; instead, it simply stably arrests their growth.

Cellular senescence is accompanied by many changes in gene expression<sup>66–68</sup>, some of which cause permanent growth arrest. For example, the cell-cycle inhibitors INK4A and WAF1 are induced (BOX 2), and the cell-cycle stimulators c-FOS, and cyclins A and B, as well as several enzymes that are needed for DNA replication, are repressed. Other changes in gene expression cause the characteristic alterations in cellular morphology and function. These changes tend to be cell-type-specific — in human fibroblasts, expression of matrix metalloproteinases, inflammatory cytokines, such as interleukin-1, and epithelial growth factors, such as heregulin, are increased, and expression of stromal matrix molecules, such as collagen and elastin, is decreased. In addition, some cells acquire resistance to apoptotic death following senescence. Together, the growth arrest, resistance to apoptosis and changes in cell functions define the cellular senescent phenotype.

Cellular senescence might also be an evolutionarily conserved process. Cells from a variety of mammals, birds and reptiles have been shown to arrest growth with a senescent phenotype under various conditions<sup>69</sup>. Moreover, ovarian stem cells from the simple organism *D. melanogaster* stably arrest growth after several divisions, and so might undergo cellular senescence<sup>70</sup>. Even single-celled organisms, notably the yeast *S. cerevisiae*, have been shown to undergo replicative senescence<sup>71</sup>. Because telomere shortening does not occur in yeast or

*Drosophila* ovarian stem cells, senescence in these cells might be due to non-telomeric stimuli, such as accumulated damage or changes in chromatin organization. Whatever the case, these examples indicate that the senescence response is certainly conserved among vertebrates, and possibly derives from cellular responses that occur in some invertebrate or unicellular organisms.

In contrast to apoptosis, little is known about whether or to what extent cellular senescence is important in simple organisms. Among complex organisms, however, there is mounting evidence that the senescence response is important for suppressing the development of cancer<sup>14,61,64,72–74</sup>. This evidence includes the fact that cancer cells almost invariably acquire mutations that prevent the senescence response<sup>10,75</sup>, and mice that carry such mutations are cancer prone<sup>76–79</sup>. These mutations tend to be those that inactivate either the p53 or RB pathways, so *in vivo* effects on apoptosis cannot be ruled out, but the activity of p53, RB or their upstream positive regulators increases in senescent cells<sup>80–88</sup>. Moreover, experimental downregulation of these activities causes cells to ignore senescence-inducing signals<sup>89–92</sup>, and experimental upregulation causes cells to arrest growth with a senescent phenotype<sup>61,93–99</sup>. As less is known about the molecules that execute the senescent phenotype than those that execute apoptosis, it has not been possible to specifically prevent cellular senescence without affecting other functions of the p53 and RB pathways. Nonetheless, the preponderance of circumstantial evidence indicates that the senescence response is a crucial tumour-suppressor mechanism.



**Figure 3 | Evolution of ageing.** Evolutionary theory holds that ageing is a consequence of the declining force of natural selection. In natural, hazardous environments, most organisms die at a relatively early age — in many cases, even before reproductive potential has declined (for example, menopause in females) — as a result of extrinsic factors (such as accidents, predators, infections and starvation). As a result, the force of natural selection declines with age. This decline, in turn, allows deleterious effects of processes that benefit early life to be retained (antagonistic pleiotropy). In protected environments, in which most extrinsic hazards have been eliminated, most organisms do not die at a young age, and the deleterious effects that have escaped the force of natural selection become prevalent in the population.

The p53 and RB tumour-suppressor pathways. Although the fate of cells is strikingly different depending on whether they undergo apoptosis or cellular senescence, they surprisingly engage the same regulatory machinery as both of these cellular responses are regulated — directly or indirectly — by the p53 and RB pathways.

p53 and RB define the two main tumour-suppressor pathways that operate in complex organisms. Both pathways comprise many upstream regulators and downstream effectors, some of which are themselves tumour suppressors (for example, INK4A and ARF, which are the products of the *CDKN2A* locus) or oncogenes (for example, *MDM2*) (BOX 2). The p53 and RB pathways interact at several points, and cross-regulate each other (BOX 2).

p53 is a transcription factor that regulates apoptosis and cellular senescence by inducing the transcription of specific genes; the RB pathway directly regulates the cell cycle and hence cellular senescence, but is also important in apoptosis — probably by interacting with the p53 pathway (BOX 2)<sup>61,93–95,100–102</sup>. It is not yet known what determines whether cells undergo apoptosis or cellular senescence in response to specific stimuli. Both responses are probably influenced by many factors, including the type and strength of the stimulus, the cell type and the tissue context.

**Evolutionary conservation of these pathways.** How did the tumour-suppressor functions of the p53 and pRB pathways evolve? p53 and RB homologues do not seem

to be present in single-celled organisms, such as yeast<sup>103</sup>. This finding indicates that p53 and RB evolved to control cellular responses that are specifically required in multicellular tissues or organisms. In the case of p53, it has been argued that its evolution provided a new regulatory module to the DNA-damage response, providing cells with the choice to proliferate, arrest or die, depending on the tissue or cell type<sup>103</sup>.

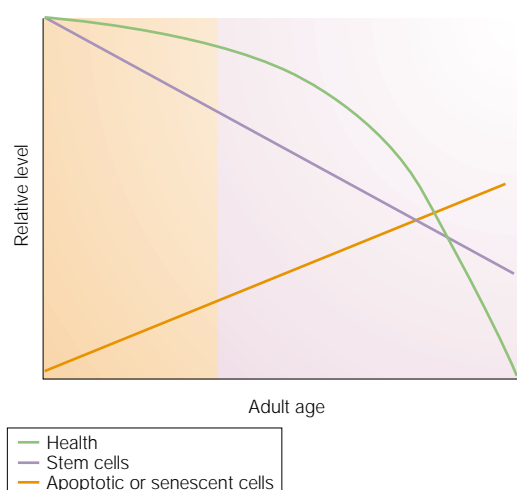
p53 and RB — as well as several components of the pathways that they control in mammals — are, however, present in simple organisms that do not develop cancer<sup>104–110</sup>. In some simple eukaryotes, the genes for p53 and RB are not only structurally related to those present in complex organisms, but they interact with the same proteins and have the same biochemical functions. For example, the *C. elegans* p53 homologue, *CEP-1*, and the *Drosophila* homologue, *dp53*, can transactivate a promoter that contains human consensus p53 binding sites. In *Drosophila*, the RB homologue, RBF, interacts with the fly E2F homologues. However, as neither *C. elegans* nor *Drosophila* develop cancer, what are the functions of p53 and RB in these organisms?

In both organisms, the p53 homologues function to induce apoptosis in response to DNA damage to the embryo or germline. In *Drosophila*, and probably *C. elegans*, RB homologues negatively control cell proliferation during embryonic development. So, the cellular processes that are controlled by p53 and RB are similar in simple and complex organisms — apoptosis and cell-cycle arrest, respectively. However, in simple organisms, these cellular processes are not tumour suppressors. Rather, they act predominantly to eliminate defective embryos or germ-cell precursors, or to sustain embryonic development. Interestingly, p53 might also help protect the adults of simple organisms from the deleterious effects of stress<sup>106</sup>, although it is not clear how p53 confers stress resistance, nor that it does so by inducing apoptosis in adult somatic cells. Whatever the case, it seems that p53 and RB, and the cellular processes that they control, have largely germline/embryonic functions in simple organisms, but acquired additional somatic functions, such as tumour suppression, in complex organisms. It seems now, however, that these somatic functions might have evolved at a cost and might not be entirely beneficial, particularly as complex organisms age. To explain how this might occur, it is important to briefly review some current ideas on why organisms age.

#### Evolution of ageing phenotypes

Evolutionary theory strongly argues that organisms are not programmed to age — that is, evolution selects for fitness, survival and reproduction<sup>20,111</sup>. Why, then, do organisms age (a decline in fitness, by definition) and die?

Organisms evolved in environments where, even if ageing did not exist, death would still occur owing to extrinsic hazards (for example, accidents, predators, infection and starvation) (FIG. 3). As an organism ages, the chance that it will die from an environmental hazard increases, which makes older organisms



**Figure 4 | Effects of apoptosis and cellular senescence on stem cells and organismal fitness with age.** During young adulthood (orange box), apoptosis and cellular senescence eliminate damaged and dysfunctional cells, which promotes the health and fitness of the organism. Eventually, however, these processes exhaust stem-cell reserves, tissue integrity and function decline, and organismal fitness is compromised. In addition, dysfunctional senescent cells accumulate, which might exacerbate the loss of tissue function and integrity.

increasingly rare in the population. Consequently, the force of natural selection declines progressively with age. This decline can have two outcomes. First, germline mutations that do not compromise fitness early in life, but do compromise fitness late in life, might not be eliminated — because the old organisms in which they act are rare or non-existent. This germline mutation accumulation theory of ageing is distinct from the damage accumulation hypothesis, which indicates that oxidative metabolism damages somatic cells, leading to ageing phenotypes. Second, because the force of natural selection declines with age, traits that benefit organisms early in life are retained, even if they have detrimental effects later in life. In natural hazardous environments, these detrimental effects are rare or not seen at all, because few or no individuals who are old enough to manifest them survive (FIG. 3). So, the detrimental effects cannot be eliminated because there are so few survivors on which natural selection can act. This is the essence of the evolutionary theory of antagonistic pleiotropy<sup>112</sup>: biological processes that are crucial for optimal development and early life fitness can — at late ages — reduce fitness by causing deleterious (ageing) phenotypes. What happens when environmental hazards are suddenly (in evolutionary time) reduced or eliminated? This, of course, is exactly what has happened among humans in the past few centuries (and among laboratory mice in the past few decades). In the less hazardous, or more protected, environment, many organisms survive far beyond the survival that is expected in the environment in which they evolved. Consequently, they show the deleterious phenotypes that have escaped natural selection.

So, are the cellular tumour-suppressor mechanisms — that is, the apoptosis and cellular senescence that occurs in adult somatic cells — antagonistically pleiotropic? These mechanisms protect organisms from cancer early in life, but might contribute to ageing phenotypes and age-related pathology later in life.

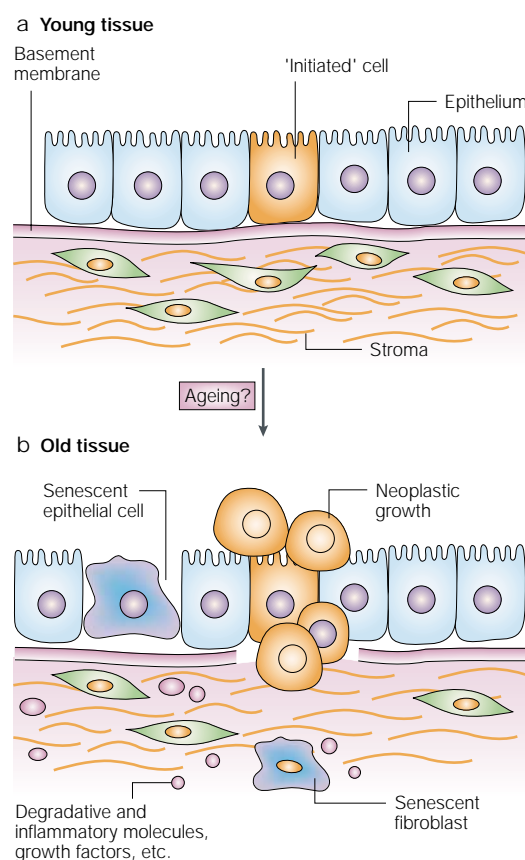
#### Apoptosis and ageing

How might apoptosis contribute to ageing? In the somatic tissues of adult complex organisms, apoptosis is important for maintaining tissue homeostasis; defective apoptosis (either too little or too much) is associated with a number of diseases<sup>113–115</sup>. In some cases, apoptosis occurs as a consequence of normal differentiation. In other cases, apoptosis eliminates damaged cells from tissues.

Cellular damage, especially from endogenous oxidative reactions, is pervasive in all cells. Damage can cause loss of function in post-mitotic cells, and there could be an advantage to eliminating such cells. For example, the elimination of dysfunctional neurons might facilitate synaptic compensation by neighbouring neurons. Loss of irreplaceable, or slowly replaceable, neurons might have little consequence for young organisms, in which synaptic plasticity can compensate for occasional cell loss. In old organisms, however, neuronal loss owing to apoptosis might outpace the compensatory mechanisms. Alternatively or additionally, neuronal apoptosis might eventually deplete progenitor- or stem-cell pools (FIG. 4), which are thought to be capable of replacing neurons in some regions of the brain or peripheral nervous system<sup>116,117</sup>. In tissues that are composed of mitotic cells, apoptosis is doubly important because damage poses the additional danger of malignant transformation. Mitotic tissues are, of course, also at risk for exhausting their supply of progenitor or stem cells. In fact, stem cells themselves can undergo apoptosis as a consequence of damage. Again, during young adulthood, the elimination of damaged cells by apoptosis would have a net positive effect. Eventually, however, stem-cell depletion would cause tissues, particularly those with a high cell turnover, to lose cellularity and, consequently, function (FIG. 4). This is, in fact, what is seen in many aged tissues<sup>118,119</sup>. Tissues might vary in how much cell loss can be tolerated before their function declines.

#### Cellular senescence and ageing

Cellular senescence, like apoptosis, might also contribute to ageing, although it could do so by two distinct mechanisms. First, because senescent cells cannot proliferate, cellular senescence, like apoptosis, might gradually deplete the renewal capacity of tissues by exhausting the supply of progenitor or stem cells (FIG. 4). Second, the senescent phenotype frequently results in secretion of degradative enzymes, cytokines and growth factors<sup>120</sup>. Moreover, senescent cells can accumulate with age, and have been detected at sites of age-related pathology<sup>121–124</sup>. These findings indicate that senescent cells can also contribute to ageing by actively disrupting the integrity, function and/or homeostasis of tissues as they accumulate.



**Figure 5 | Model for how senescent cells might promote cancer.** **a** | A prototypical young tissue is shown. The tissue is composed of an epithelium in contact with a basement membrane, which is maintained by the underlying stroma that contains resident fibroblasts. Oncogenic mutations can occur in the cells of young tissues ('initiated' cell), but the tissue environment suppresses the expression of its potential neoplastic phenotype. **b** | With age, senescent cells accumulate and secrete factors (such as degradative enzymes, inflammatory cytokines and growth factors) that disrupt the tissue structure, which, in turn, allows the 'initiated' cells to express their neoplastic phenotypes.

It is not known why senescent cells, particularly senescent stromal cells (fibroblasts), secrete the factors they do. The senescent secretory phenotype could simply be an unselected byproduct of the growth arrest, or it might be adaptive — for example, it might prime non-senescent neighbouring cells to proliferate when there is a need for cell replacement or tissue repair. Whatever the case, the phenotype of senescent stromal cells resembles that of activated, or carcinoma-associated, fibroblasts<sup>125,126</sup>, which are believed to promote a tissue microenvironment that facilitates the development of cancer (BOX 1). These similarities indicated that senescent stromal cells might actually promote cancer progression as they accumulate (FIG. 5). This possibility might seem paradoxical, given the evidence that cellular senescence is a tumour-suppressor mechanism. However, it is consistent with the idea that the senescence response is antagonistically pleiotropic.

Recent findings indicate that senescent fibroblasts can indeed stimulate the growth and tumorigenic transformation of premalignant epithelial cells in culture and *in vivo*<sup>127</sup>. These data raise the possibility that, whereas cellular senescence protects organisms from cancer early in life, it could promote cancer progression later in life. As discussed earlier, both mutations and a permissive microenvironment are needed for cancer to develop (BOX 1). So, the exponential rise in cancer that occurs with age (FIG. 1) might result from two synergistic processes: the acquisition of oncogenic mutations, which can inactivate tumour-suppressor mechanisms, and relaxed control by the tissue microenvironment, owing to the presence of senescent cells (FIG. 5).

**Molecular links: tumour suppression and ageing**  
 What genes are responsible for the antagonistic pleiotropy of apoptosis and cellular senescence? Answers to this question are just beginning to emerge, and they focus on p53.

Three groups recently created mice in which p53 expression or activity was higher than normal<sup>128–130</sup>. These mice do not exist in nature, but have provided valuable insights into effects of p53 that, in some cases, would not have been obvious from studying wild-type or *Trp53*<sup>-/-</sup> mice. Consistent with p53's role as a tumour suppressor, all three transgenic mouse lines had a much lower incidence of cancer. Cancer is a significant cause of death in mice, as it is in humans. But, surprisingly, these mice did not live longer. In two of the three lines, lifespan was shorter.

In the best-characterized line, a spontaneous recombination event resulted in the deletion of the upstream region and six exons from one of the p53-encoding alleles<sup>128</sup>. The resulting amino-terminally truncated mutant p53 protein (p53m) is thought to form a complex with the wild-type p53 produced by the other allele and enhance its activities. p53<sup>m/+</sup> mice had substantially less cancer; however, they also had a 20–30% shorter lifespan. Moreover, these animals showed several signs of premature ageing, including tissue atrophy (such as in skin, skeletal muscle, liver and lymphoid organs), osteoporosis, poor wound healing and sensitivity to stress. Similar results were found with mice that constitutively express a transgene that encodes a p53 protein with a slightly different amino-terminal truncation, although this report is still preliminary<sup>130</sup>. A third mouse line carried an extra copy of the normal *Trp53* gene that included many kilobase pairs of upstream DNA<sup>129</sup>. These p53-tg mice were also significantly cancer resistant, but they showed no signs of premature ageing. However, despite their lower incidence of cancer, they did not seem to live longer than wild-type mice.

What might be responsible for the differences between p53<sup>m/+</sup> and p53-tg mice? The authors speculate<sup>119,129</sup> that the difference lies in the way the excess p53 activity is regulated. p53<sup>m/+</sup> mice constitutively express the mutant p53 protein, and so p53 activity is constitutively high in these animals. By contrast, p53-tg mice regulate the extra *Trp53* copy normally; so, p53 activity is abnormally high only when induced by damage or



other stimuli. The cancer resistance of p53-tg mice could be due to their heightened p53 damage response. Alternatively, however, it might be due to the fact that p53-tg mice must acquire an additional genetic change to inactivate the additional copy of *Trp53*. Both the cancer resistance and premature ageing shown by p53<sup>tm/+</sup> mice could be due to excessive p53-dependent apoptosis, which would eliminate potential cancer cells but also deplete renewable tissues of stem cells<sup>119</sup>. Alternatively, the p53<sup>tm/+</sup> ageing phenotypes could be due to excessive cellular senescence, and subsequent loss of tissue integrity and function. In this case, cellular senescence might fail to promote late-life cancers because the cancer-promoting effects are limited to premalignant cells<sup>127</sup>, which could be efficiently eliminated or arrested in p53<sup>tm/+</sup> mice.

Implications for controlling cancer and ageing  
The intertwined relationships between tumour suppression and longevity have interesting and important implications for the limits and promise of interventions that are aimed at preventing or postponing cancer and ageing. On one side of the spectrum, the phenotypes of organisms that are defective in certain DNA-repair genes indicate that some tumour-suppressor mechanisms — those that are involved in genomic maintenance — also

promote longevity. So, strategies that prevent DNA damage (such as heightened antioxidant defences) or improve DNA-repair mechanisms are likely to suppress both cancer and ageing phenotypes. Will it be possible to improve genomic maintenance systems, given their complexity and numerous interactions with the cellular transcription, replication and cell-cycle machineries? This, of course, remains to be seen. On the other side of the spectrum, antagonistic pleiotropy predicts that it might be difficult, if not impossible, to improve cellular tumour-suppressor mechanisms without accelerating ageing, and *vice versa*. Mitigating against this dire view are the p53-tg mice. It is still possible that, following further characterization, these mice will show some signs of premature ageing, as it might be expected that they have an increased average lifespan as a result of their greatly decreased susceptibility to cancer. However, taken at face value, the phenotype of these mice indicates that enhanced — but regulated — p53 activity might offer improved tumour suppression without accelerated ageing. Needless to say, much more work is needed before we know whether or to what extent any of these possibilities is really feasible. Nonetheless, as modern cancer and ageing research converge, chords of both caution and optimism resound!

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